

AN AUTORADIOGRAPHIC STUDY OF THE UTILIZATION OF FREE EXOGENOUS AMINO ACIDS BY STARFISHES¹

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In a previous report (Ferguson, 1967) evidence was provided that at least two very different species of starfishes are capable of making extensive use of dissolved amino acids and glucose which might occur in their environment. That paper confirmed results of earlier experiments performed by Stephens and Schinske (1961), demonstrating that these two starfishes, along with many other types of invertebrates, can remove glycine from their surrounding medium. In addition, it showed that in most cases the dissolved nutrients taken up by these forms are retained almost exclusively in body wall components and do not appear to be translocated into the internal organs.

As that study was based on quantitative measurements of labeled material taken up into the different major regions of the body, it could not reveal the precise cellular location of uptake, or possible translocations of the absorbed nutrients within the regions studied. However, it was inferred, largely on the basis of previous autoradiographic observations of animals fed clams containing labeled nutrients (Ferguson, 1963a, 1963b), that much of the epidermis was involved in the absorptive process.

The present investigation was undertaken in order to locate more precisely the significant areas in which the exogenous nutrients are taken up, and to determine the normal limits of distribution of these nutrients within the body. The observations have been limited to the localization of absorbed amino acids, since these compounds do not appear to be ingested to any significant extent under the experimental conditions that have been employed. As shown previously (Ferguson, 1967), dissolved sugars are sometimes taken up orally. For that and other reasons their uptake presents a considerably more complex picture, which will require further analysis.

MATERIALS AND METHODS

The starfishes used in these experiments were freshly collected specimens of *Asterias forbesi* and *Henricia sanguinolenta*, both from the Woods Hole region, and *Echinaster spinulosus*² from Tampa Bay, Florida. Only individuals about 3

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² After surveying the available literature and consulting with the curators of the echinoderm collection of the Marine Laboratory of the Florida Department of Conservation (who have been in communication with the U. S. National Museum on the problem), it is evident that the taxonomy of the echinasters is at the present time in a state of considerable confusion. The forms used here were all obtained from an apparently homogeneous population located on tidal grass flats near the east side of the southern end of the Sunshine Skyway bridge.



FIGURE 1. Autoradiograph of a section through the mid-region of a ray of a specimen of *Asterias* sacrificed 1 hour after being placed in C^{14} -labeled amino acid medium. Darkened epidermis indicates uptake of the labeled amino acids into that region. No significant radioactivity is detectable in other areas. This preparation, like those shown in all of the following figures, was unstained. 12 $\times$.

FIGURE 2. Autoradiograph of *Asterias* ray similar to Figure 1, but this specimen was sacrificed after 8 hours in the medium. While incorporation of the labeled amino acids has been somewhat greater, the radioactive areas are essentially the same as those in Figure 1. 12 $\times$.

FIGURE 3. Equivalent autoradiograph of a sectioned ray of *Asterias* incubated 8 hours in the labeled amino acid medium and then placed in circulating sea water for 3 days before sacrifice. The tracer has not moved from the epidermal areas in which it was initially taken up. 12 $\times$.

to 5 cm. in diameter were selected for study. For the Woods Hole species, each animal was placed in 50 ml. of filtered sea water to which had been added 0.5 microcurie (0.0033 mg.) of a mixture of 15 uniformly labeled L-amino acids (representing a synthetic algal protein hydrolysate mixture, manufactured by the New England Nuclear Corp. of Boston, Mass.). One group of specimens was left in the medium for 1 hour and then rinsed in sea water and sacrificed. The remaining animals were left in the medium for 8 hours before being placed in fresh sea water. Of these latter specimens, some were sacrificed immediately while other groups were placed for 3 and 20 days in circulating sea water before being sacrificed. The last two groups were allowed to feed *ad libitum* on small clams during their periods of retention.

In the case of the specimens of *Echinaster*, 6 individuals were placed in 100 ml. of filtered sea water containing approximately 4.0 microcuries of uniformly labeled glycine-C¹⁴ in a 0.1 mM solution. Some individuals were removed from this medium and sacrificed after 1, 6, and 24 hours. The specimens remaining after 24 hours were rinsed and placed in tanks of uncontaminated sea water for periods of 3, 9, and 20 days.

Sacrifice was accomplished by severing the rays from the disk and cutting each of them transversely into equal portions. The pieces obtained were then fixed for 3 days in Bouin's solution diluted 50% with water (to reduce the rate of decalcification). They were then dehydrated through alcohols, cleared in xylene, and embedded in paraffin in a vacuum oven. Sections of the material were cut at 10 microns thickness, mounted on "subbed" slides, deparaffinized, rehydrated, and covered with AR-10 stripping film as recommended in the directions for use of the product as supplied by the Eastman Kodak Co. After air-drying, the slides were kept in black plastic boxes in a refrigerator for up to 6 months and then developed 20 minutes in Kodak D-19 developer. After fixing, the preparations were rinsed, dehydrated in alcohol, cleared in xylene, and mounted with a coverslip, using a diluted synthetic medium. In most cases, staining was not employed.

This procedure, of course, retains and makes visible only the labeled amino acids which have become firmly bound up in the tissues. As was previously shown (Ferguson, 1967), this actually represents a rather sizable proportion of the total amino acid taken up by the forms. In the case of *Asterias*, for example, the bound fraction was found to range from approximately 65% in 1-hour specimens to 90% in those kept 20 days.

RESULTS

Darkened areas of the emulsion of the stripping film covering the sections demonstrated that all the animals had taken up and retained significant amounts of the labeled amino acids which were provided in their medium. The proportion of the amino acids taken up cannot be stated with certainty as the method is not quantitative. The counting of a few aliquots of medium before and after the

FIGURE 4. Section of *Asterias* ray similar to that in Figure 3, but kept 20 days in circulating sea water. There are only minor differences between the distribution of the tracers in this specimen and the previous ones. These differences stand out more clearly in Figures 13-15, 12×.

FIGURES 5 AND 6. Preparations similar to those in Figures 3 and 4, but of *Henricia*. Results are much the same except that the tissues show up more distinctly because of their natural coloration. Both 12×.

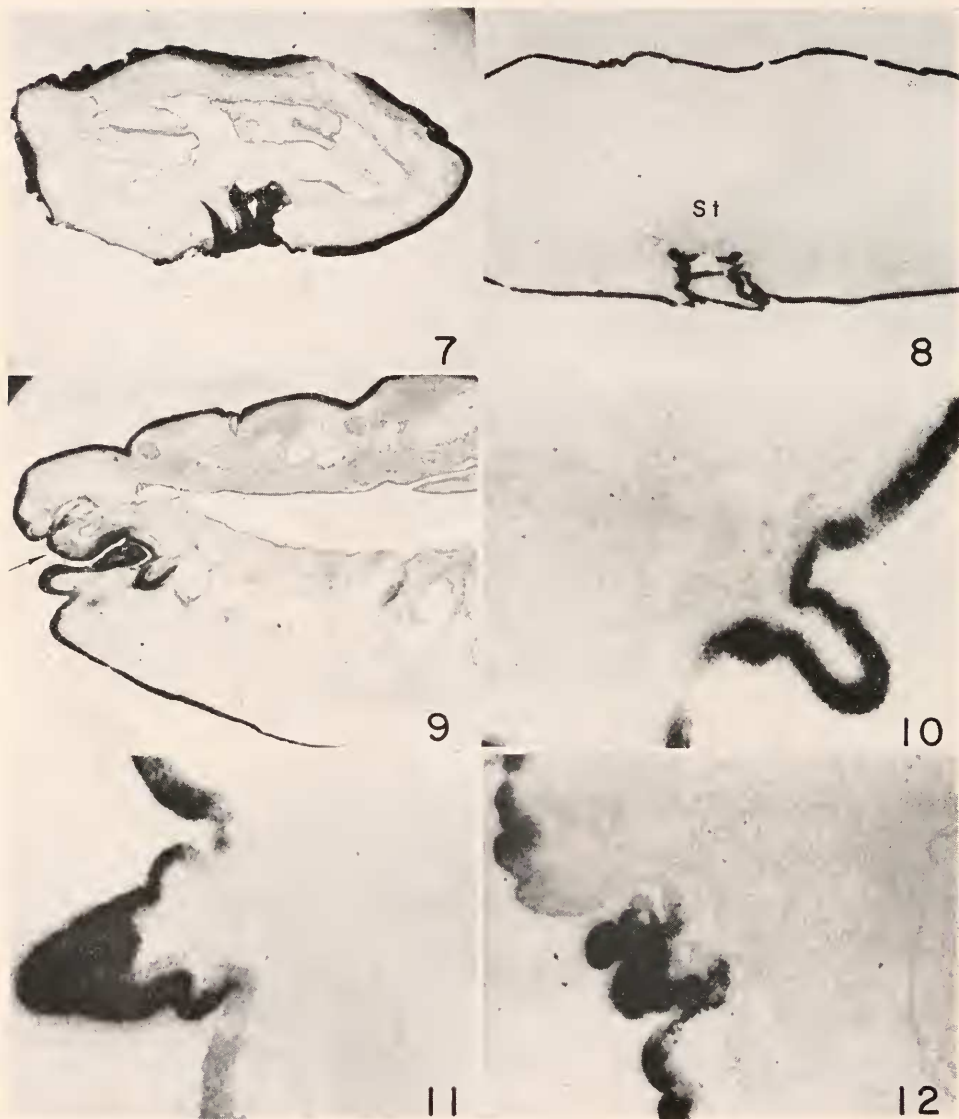


FIGURE 7. Autoradiograph of specimen of *Echinaster* placed for 24 hours in a medium containing glycine- C^{14} , and then kept in sea water for 20 days before sacrifice. As with the other species, the labeled amino acid has been incorporated into the epidermal regions. Other tissues stand out due to their natural coloration. Greater detail of the ambulacral area is shown in Figure 17. $14\times$.

FIGURE 8. Autoradiograph of transverse section through disk of a specimen of *Echinaster* sacrificed 6 hours after being placed in glycine- C^{14} medium. While the epidermis has incorporated a great quantity of the labeled glycine, apparently none of it has entered the mouth and been taken up by the stomach or other tissues. (St, area of stomach tissue.) $8\times$.

FIGURE 9. Autoradiograph of a longitudinal section of a ray tip of a specimen of *Echinaster* sacrificed 24 hours after being placed in medium containing glycine- C^{14} . Arrow indicates optic

experiments, however, indicated that anywhere from about 30 to 60% of the tracer was removed by the different animals. Doubtless, size and species differences were the main factors involved in the variation.

General observations

In each of the 3 species, the labeled amino acids were taken up and retained primarily by the epidermal cells forming the body surface. This can be seen most clearly in Figures 1-9, which are low-magnification views of typical sections. The greatest uptake appeared to occur in the tube feet, papulae, and some of the spines, but apparently all external areas were involved to some extent. Several workers, including Gislén (1924) and Budington (1942), have described ciliary currents maintained over the surfaces of starfishes, and very likely the intensity of these currents, as well as the degree of exposure of the structures, were significant in determining the quantity of the amino acids taken up by the different regions.

While carbon-14 is considered a low-energy β -particle emitter, the energy of the particles is nevertheless too great to permit precise intracellular localizations. The resolution in the present experiments was probably on the order of about 5 microns. Even at this resolution, it could be observed that the amino acids were rapidly distributed throughout the cytoplasm of the elongated epidermal cells. This fact is illustrated in Figure 10, which shows a portion of a specimen of *Henricia* after a single hour of exposure. The radioactivity is apparently no greater in the external border of the cells than in the basal regions.

In this same figure, as well as in Figures 11 and 12, depicting equivalent areas of specimens of *Asterias* at 8 hours and 20 days, respectively, it can be seen that practically all of the epidermal cells participated in the uptake process. A few lighter regions in the layer, representing areas of less activity, may be observed, however. The significance of these is not certain, but since they generally appear to merge gradually with denser areas, they probably are portions of the epidermis which were more protected from the ciliary currents. Smaller, more distinct areas of lightness, as in the upper portion of Figure 10, can usually be traced in adjacent sections to the larger patches.

Close observation of the epidermis in many cases revealed slight irregularity in uptake of amino acids by the various types of cells making up the layer. The limitations of resolution made it difficult to correlate this unevenness with the different cells in the layer, but after many observations it seems probable that glandular cells tended to accumulate more radioactivity than other types. These differences were relative, since the actual amount of amino acid taken up by any cell chiefly depended on the region in which it was located.

In addition to these variations in the intensity of the label incorporated into

cushion, which took up an appreciable quantity of the labeled amino acid, as did the remainder of the epidermis. The optic cups stand out as small light areas in the optic cushion, because they incorporated little of the tracer. 30 $\times$.

FIGURE 10. Autoradiograph of a typical section through a papula. *Henricia* after 1 hour of incubation with the labeled amino acids. 90 $\times$.

FIGURE 11. Preparation similar to that in Figure 10. *Asterias* after 8 hours of incubation with the labeled amino acids. 125 $\times$.

FIGURE 12. Similar to Figure 11, but specimen kept 20 days after exposure to the labeled amino acids. Note that the radioactivity remains confined exclusively in the epidermis. 85 $\times$.



FIGURE 13. Autoradiograph similar to that in Figure 1 (*Asterias*, 1 hour). Radioactivity is found in epidermis of tube feet and cellular area of radial nerve cord. (H, location of hemal septum; N, radial nerve cord; Tf, tube foot.) 125 $\times$.

FIGURE 14. Autoradiograph similar to Figure 3 (*Asterias*, 3 days). Small amounts of radioactivity are found in deeper layers of radial nerve cord and tube feet. (R, radial canal of water vascular system; P, perihemal sinus.) 85 $\times$.

FIGURE 15. Autoradiograph similar to Figure 4 (*Asterias*, 20 days). Some radioactivity is found in deeper layers of radial nerve cord, and in inner layer of tube foot (arrow). Darkening of radial canal is due to artifact of fixation. (Mu, lower transverse ambulacral muscle.) 125 $\times$.

the surface tissues, gaps may be seen in the epidermal layers shown in some of the figures. These are artifacts caused by various types of damage suffered by the rather delicate layer during preparation of the sections. Also, chemical reduction of the photographic emulsion could be detected in a number of instances. It most commonly occurred over osteocytes, some amoebocytes, and various scattered cells in the digestive system. Such areas could easily be recognized from the fact that they were as common in the 1-hour specimens as those kept 20 days, and the distribution of the silver grains was somewhat different from those produced by radiation.

Observations on specific areas

A number of areas in the different preparations were selected for more detailed study. In general, the observations revealed very little significant variation among the three species in the reaction of these regions to the presence of the labeled amino acids in the medium.

Mouth and stomach. These regions are most clearly shown in Figure 8, which represents a section of a specimen of *Echinaster* after 6 hours' exposure to the glycine- C^{14} . In addition, stomach regions of specimens of *Asterias* sacrificed 3 and 20 days after exposure to the labeled amino acid mixture may be seen in Figures 19 and 21. In all cases, the epidermis extending to and covering the buccal membrane readily took up the amino acid, but practically none of the labeled material worked its way through the esophagus into the stomach. No significant amounts of radioactivity were detected in any portion of the digestive system.

Ray tip. The epidermis on the tips of the rays took up at least as much of the labeled amino acids as did the epidermis of other regions. As can be seen in the specimen of *Echinaster* (exposed 24 hours) shown in Figure 9, the terminal tentacle and the region of the optic cushion were particularly radioactive. The optic cups, which apparently were deficient in cytoplasmic material (Smith, 1937), stand out as lighter areas in the optic cushion.

Ambulacral groove. Because of the presence of many important structures, the region of the ambulacral groove was closely scrutinized in all the specimens. Typical results are shown in Figures 13-17. The amino acids were very readily taken into the epidermal layers of both the sides and sucker portion of the tube feet. Considerable activity was also found in the radial nerve cord, mostly localized in the basal cellular layer, but present to some degree in the fibrous area. In addition, small but possibly significant traces of radioactivity could be discerned in adjacent tissues, especially in specimens kept for the longer periods of time. Such activity

FIGURE 16. Preparation similar to those in Figures 13, 14 and 15, but of *Echinaster* after 24 hours of exposure to glycine- C^{14} . 85 $\times$.

FIGURE 17. Specimen of *Echinaster* similar to that in Figure 16, but kept additional 20 days in sea water. Note that a small portion of the large amount of glycine- C^{14} taken up by this animal has found its way into the deeper areas of the nerve cord and the tube feet. A clump of radioactive cells (amoebocytes?) in apical end of lower tube foot are especially conspicuous. 85 $\times$.

FIGURE 18. Autoradiograph of a transverse section through edge of the madreporite and stone canal of a specimen of *Echinaster* after 6 hours exposure to glycine- C^{14} . None of the labeled glycine has penetrated into the water vascular cavity. (M, madreporite; S, location of stone canal.) 85 $\times$.



FIGURE 19. Autoradiograph of a transverse section through the madreporite region of a specimen of *Asterias* 3 days after exposure to the labeled amino acids. Considerable uptake has taken place in the outer epidermal areas of the madreporite, but no significant radioactivity is evident elsewhere. (M, madreporite; S, location of stone canal; A, location of axial gland; St, area of stomach tissue.) 33 $\times$.

FIGURE 20. Similar to Figure 19, but this specimen was kept 20 days. (S, location of stone canal.) 30 $\times$.

FIGURE 21. Different region of the same specimen as in Figure 20. (St, area of stomach tissue; S, location of stone canal.) 30 $\times$.

FIGURE 22. Autoradiograph of a transverse section through a ray of a specimen of *Henricia* after 8 hours exposure to labeled amino acids. Large epidermal glands (right) may be seen

was seen in Lange's nerve (the motor neural complex), the hemal septum, and the aboral lining of the perihemal canal. Only background reduction of the emulsion could be detected in the various muscles, connective tissue, and water vascular canals in this region. The lateral motor centers described by Smith (1950), located in the epidermis juxtalateral to the bases of the outer rows of tube feet, took up relatively little amino acid, possibly because of their deep, sheltered position.

Madreporite, stone canal, and axial gland. While not without controversy (cf. Nichols, 1966), it is probable that the madreporite and stone canal serve as structures which bring sea water into the water vascular system for ultimate use in the protraction of the tube feet and, by pressure-filtration through the ampullae, for replenishment of the coelomic fluid. If sea water does enter these structures, they could possibly also be significant routes for the uptake of exogenous dissolved nutrients and the distribution of organic materials to the interior regions of the oral body wall. Figures 18–21 illustrate the observations made on these structures.

In every case the radioactivity was confined to the most exposed regions of the madreporite epidermis. No significant activity could be observed in sections through any level of the stone canal, the axial gland, or the tissues lining the perihemal sinus surrounding the axial gland. At least three different possibilities could explain these results. First, there may have been no flow of medium through the structures. Even though the animals were confined to small containers during their exposure to the amino acids, this possibility seems unlikely since the tube feet generally remained quite active and presumably created at least some demand on the water vascular channels for water. Second, the internal tissues may not have possessed an affinity for the labeled amino acids. In no previous study, however, have starfish cells failed to exhibit uptake of these materials. Third, the amino acids could have been completely removed from the inflowing water by the superficial tissues of the madreporite. This final hypothesis appears to be the most reasonable.

Large epidermal glands. The possible significance of small glandular cells in producing irregularities in the intensity of radioactivity in the epidermis has already been mentioned. In *Henricia* and *Echinaster* there are additional very large glands which extend deep into the connective tissue of the body wall. These apparently produce a copious, mucus-like secretion. These glands are of special interest as they must present a significant demand for nutrients in an area somewhat distantly removed from the presumed major source of supply, i.e., the coelomic fluid (cf. Ferguson, 1964a, 1964b).

The large epidermal glands could readily be recognized in the sections (Figs. 22–24). In a number of the specimens incubated for the shorter periods of time, the tracer was seen to have penetrated at least part way down into the glands (Fig. 22). In the specimens maintained for a number of days after they were

extending down into body wall connective tissue, and radioactivity has penetrated into the glands. (DG, location of digestive gland.) 85 $\times$.

FIGURE 23. Similar to Figure 22, but specimen kept additional 3 days in circulating sea water. Contents of large epidermal gland is seen being extruded. Some radioactivity is found in this material. 85 $\times$.

FIGURE 24. Large epidermal gland in a specimen similar to that in Figure 7 (*Echinaster*, 21 days). Most of the radioactivity has left the gland, which is seen filled with refractile particles. 360 $\times$.

removed from the labeled medium, however, only small quantities of the radioactive material could be detected in these structures. Little is known of the chemical nature of the secretion of the glands. Hyman (1955) refers to it simply as "gelatinous." The present observations, while admittedly inconclusive, do suggest that at least part of the material is derived from external amino acids sources.

Translocation of epidermally absorbed nutrients

Translocation of the epidermally absorbed amino acids from the epidermal cells to other regions appears to be very limited. This observation is illustrated in Figures 1-4, representing comparable transverse sections through the medial portion of the rays of *Asterias* specimens sacrificed after different periods of time. The distribution of radioactivity in the 20-day specimen (Fig. 4) is almost identical to that in the 1-hour specimen (Fig. 1). In none of the preparations can significant amounts of the tracers be detected in the digestive glands, ampullae, ossicles, connective tissue, and most other parts. The same observation holds true for the two transverse sections of *Henricia* (Figs. 5 and 6), representing 3- and 20-day specimens. Natural coloration in these animals makes their tissues stand out more clearly in the photographs. A 21-day specimen of *Echinaster* may be seen in Figure 7, and, again, nearly all of the tracer remained localized in the epidermis. Figures 10-12 are more highly magnified views and clearly illustrate the sharp demarcation, even after 20 days, between the epidermal layers retaining the labeled material and the underlying connective tissue in which is found only normal background activity.

A few cases were seen, however, which could possibly be interpreted as slight degrees of translocation. A gradual increase in radioactivity of some of the oral perihemal areas has already been mentioned. In addition, a small inward movement of the tracer could also be detected in many of the more heavily exposed tube feet (Figs. 15 and 17). Most of this activity became localized in the peritoneal cells lining the lumina of these structures or in a clump of cells, possibly amoebocytes, usually found in the lower apical ends of the cavities. In any case, the labeled nutrient finding its way into these places probably could not represent more than a small fraction of a per cent of the total amino acid taken up by the adjacent epidermis.

DISCUSSION

The results of the foregoing experiments seem to illustrate a considerable dependence of the starfish epidermal cells on soluble amino acid sources in the external environment. There are no areas of the body surface that do not appear to possess this property. While some variation in uptake was noted among the different cell types which make up the epidermis, and among the different body regions, large quantities of the labeled amino acids were taken up everywhere the medium came in contact with the animals.

Relatively little is known about the mechanism by which these substances are absorbed into the cells. The autoradiographs produced no indication of significant uptake of the compounds on the cell surfaces, but rather, revealed that the substances were taken in rapidly (at least in less than an hour) through the cell membranes and evenly distributed throughout the cytoplasm of the cells. Further-

more, as only insoluble materials are visualized by the technique, much of the absorbed amino acid must have also been converted, with equal rapidity, into polypeptides or other compounds. It is possible that this conversion is the driving force of a "facilitated diffusion" of the compounds into the cells, and that no other "active" mechanisms are necessary for transport. Such an hypothesis, however, is at the present time rather difficult to defend, since it may be calculated from previous results (Ferguson, 1967) that a suitable concentration gradient for entry would not appear to exist.

Possibly further evidence against a diffusion mechanism as a significant factor in the uptake process is provided by the fact that the acellular optic cups stood out as areas of little uptake, while the cellular areas surrounding them demonstrated great affinity for the tracers. Perhaps the optic cups are relatively impermeable to the amino acids, but more likely, they lack the specific absorptive machinery necessary for the maintenance of the process.

The almost universal failure of the absorbed amino acids to move out of the epidermal cells into other regions of the body probably is a point of great functional significance. It might imply that there exists some sort of barrier beneath the epidermal cells that would bar most of the inward migration of the amino acids. Such a barrier, if it existed, presumably would also reduce movement of metabolites from inside the body outward to the epidermal cells, and would leave the epidermal cells to subsist in a fairly autonomous fashion at the surface of the animal. This barrier could have its greatest importance as a mechanism in preventing the loss of nutrient pools maintained in the body fluids.

Close examination of some of the observations reported in this investigation, however, makes the existence of a significant barrier of this kind quite unlikely. First of all, there were a few areas, such as in the tube feet, in which small but distinct quantities of the tracers did appear to migrate inward. Secondly, the inability of the labeled amino acids in the medium to penetrate through the outer regions of the madreporite, buccal membrane, and other areas indicates an extremely strong affinity on the part of the epidermal cells for the uptake and retention of the compounds. This affinity of the epidermal cells for the exogenous amino acids is so great, it seems likely that they are also capable of drawing on any nutrient pools which might tend to form in the ground substance of the underlying connective tissue. In this view, the epidermal cells attract and absorb amino acids from all directions. Such action causes diffusion gradients to be established in the connective tissue layers, and these gradients are slanted towards the epidermal cells except when overdriven by large quantities of substances taken up from the external medium.

If this hypothesis is correct, it is the epidermis itself which is the primary barrier against both the movement of externally absorbed compounds into the deeper regions of the body, and the loss to the environment of nutrient pools maintained in the body fluids. Actually, it is unlikely that more than very small amounts of nutrients normally reach the epidermis from internal sources. The concentration of amino acids maintained in the coelomic fluid are very low (Ferguson, 1964a) and the diffusion distance, through the connective tissue of the body wall, is relatively great. Even with complete removal of the amino acids by the epidermal cells, the gradient of diffusion toward these cells would be in most cases quite

slight. The concentrations of amino acids expected in the immediate micro-environment of the animals generally would be a much more suitable source of supply.

It may be noted further that the modified epidermal tissue forming the lateral motor complexes, which were found to be somewhat protected from the external sources of amino acids, are bordered on their basal side by extensions of the perihemal sinus. These in turn are separated by only a tenuous tissue layer from the visceral coelomic cavity. The lateral nervous centers are thus in a position to receive nutrients *via* an internal route by mechanism previously described (Ferguson, 1964b). As extensions of the perihemal sinus can also be traced to the vicinity of most of the major muscles in the oral side of the body, such as the lower, transverse ambulacral muscle shown in Figure 15, this cavity is probably a most important route for the distribution of endogenous nutrients within that portion of the body.

It is concluded, then, that while starfishes generally appear to make extensive use of exogenous sources of free amino acids, these are of negligible importance to most of the tissues of the body, and in actuality, they only benefit the epidermis and some of the structures immediately derived from it. This benefit, however, appears to be very significant, and probably in many cases represents the chief source of nutrition for these cells. Underlying cellular regions are generally situated in close proximity to various body cavities from which the cells presumably obtain nutrients distributed from endogenous sources.

SUMMARY

1. Specimens of *Asterias forbesi*, *Henricia sanguinolenta*, and *Echinaster spinulosus* were exposed up to 24 hours to sea water media containing C¹⁴-labeled amino acids. The distribution of the labeled compounds after periods of 1 hour to 21 days was determined by means of stripping-film autoradiographs of histological sections of the specimens.

2. The results from all three species were very similar. Large quantities of the supplied amino acids were found to have been taken up and retained by the epidermal cells in almost all regions of the body, and especially in those parts most in contact with the external medium. Variations in uptake between the different types of cells making up the epidermal layer appeared to be only minor and relative.

3. The amino acids were found not to have penetrated through the madreporite channels or through the buccal opening. If fluid entered these regions, the amino acids were presumably completely removed by the adjacent superficial cells, which always demonstrated large amounts of uptake.

4. Secretions by the large epidermal glands characteristic of *Henricia* and *Echinaster* appeared to contain at least some material derived from the exogenous amino acids supplied.

5. There was very little evidence of movement of the absorbed amino acid out of the epidermal cells within the 3-week period during which the observations were continued. Only slight translocation occurred in some of the tube feet and in the region of the radial nerve cord.

6. It is concluded that absorption of environmental amino acids (and probably other compounds) by the epidermis is an important and often principal source of

nutrition for the cells making up this tissue; other areas of the body, however, depend almost exclusively on endogenous sources of nutritive substances, probably delivered by the fluids circulating through the various body cavities.

LITERATURE CITED

- BUDINGTON, R. A., 1942. The ciliary transport-system of *Asterias forbesi*. *Biol. Bull.*, **83**: 438-450.
- FERGUSON, J. C., 1963a. The physiological mechanisms of nutrient transport in the starfish, *Asterias forbesi*. Doctoral dissertation, Cornell University.
- FERGUSON, J. C., 1963b. An autoradiographic study of the distribution of ingested nutrients in the starfish, *Asterias forbesi*. *Amer. Zool.*, **3**: 542.
- FERGUSON, J. C., 1964a. Nutrient transport in starfish. I. Properties of the coelomic fluid. *Biol. Bull.*, **126**: 33-53.
- FERGUSON, J. C. 1964b. Nutrient transport in starfish. II. Uptake of nutrients by isolated organs. *Biol. Bull.*, **126**: 391-406.
- FERGUSON, J. C., 1967. Utilization of dissolved exogenous nutrients by the starfishes, *Asterias forbesi* and *Henricia sanguinolenta*. *Biol. Bull.*, **132**: 161-173.
- GISLÉN, T., 1924. Echinoderm studies. *Zool. Bidr. Uppsala*, **9**: 1-316.
- HYMAN, L. H., 1955. The Invertebrates: Vol. IV, Echinodermata. McGraw-Hill, New York.
- NICHOLS, D., 1966. Functional morphology of the water-vascular system. *In: Physiology of Echinodermata*, R. A. Booloottian, Ed. Interscience Publishers, New York.
- SMITH, J. E., 1937. On the nervous system of the starfish *Marthasterias glacialis* (L.). *Phil. Trans. Roy. Soc. London, Ser. B*, **227**: 111-173.
- SMITH, J. E., 1950. The motor nervous system of the starfish, *Astropecten irregularis* (Pennant), with special reference to the innervation of the tube feet and ampullae. *Phil. Trans. Roy. Soc. London, Ser. B*, **234**: 521-558.
- STEPHENS, G. C., AND R. A. SCHINSKE, 1961. Uptake of amino acids by marine invertebrates. *Limnol. and Oceanog.*, **6**: 175-181.